AN EXPERIMENTAL-NUMERICAL STUDY OF SMALL SCALE FLOW INTERACTION WITH BIOLUMINESCENT PLANKTON

Michael I. Latz
Marine Biology Research Division
Scripps Institution of Oceanography
La Jolla, CA 92093
(619) 534-6579
mlatz@ucsd.edu

James Rohr
Naval Command Control & Ocean Surveillance Center
Code 574
San Diego,CA 92152-6040
(619) 553-1604
rohr@nosc.mil

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LONG TERM GOALS

Bioluminescence is one of the most cosmopolitan organism behaviors in the marine environment, found in all oceans of the world at all depths. My long-term goals are to understand the hydrodynamic conditions which stimulate bioluminescence, the environmental and physiological factors which regulate the amount of light emission, and ultimately the roles of bioluminescent displays in biological interactions and the structuring of marine communities.

OBJECTIVES

The goal of this study is to couple numerical and experimental approaches to investigate the effects of quantified levels of flow stimuli on bioluminescence excitation at the small length and time scales appropriate for individual plankton. This interdisciplinary project is a collaboration involving Jim Rohr at NRaD San Diego (experimental fluid mechanics), and Said Elghobashi at UC Irvine (computational fluid mechanics). We consider bioluminescence the most sensitive tool for examining organism response at these scales.

APPROACH

<u>Response to shear and acceleration.</u> We have coupled computational and experimental approaches to investigate the response of dinoflagellates to local conditions of shear and acceleration in different flow fields. The flashing of luminescent dinoflagellates is used as an instantaneous monitor of organism response; flash location and instantaneous velocity allows us to pinpoint the location of the cell within the flow field. Two flow fields which have been investigated are fully developed pipe flow, and the developing flow in a 4:1 convergence.

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The computational study calculates all the properties of the flow field by solving the full, incompressible Navier-Stokes equations. Therefore the levels of shear, shear stress, acceleration, extensional stress, and pressure are known for each position in the flow field. The stimulatory properties of the flow field can be determined by correlating the computational results with the experimental data on organism responsiveness.

Two species of dinoflagellates, *Lingulodinium polyedrum* (=*Gonyaulax polyedra*) and *Ceratocorys horrida*, have been studied. Because *C. horrida* is approximately one order of magnitude more sensitive than *L. polyedrum* based on pipe flow studies, we can directly test if cells are responding to boundary layer shear stress levels.

<u>Mechanism of the shear response.</u> When dinoflagellates respond to fluid shear, are they sensitive to the force acting on them (shear stress) or the rate of strain acting across the cell (shear rate)? Graduate student Jennifer Nauen has examined this aspect of bioluminescence stimulation by manipulating the fluid viscosity in fully developed pipe flow. Because shear stress = shear rate x viscosity, the shear stress acting on the cell can be increased by increasing the fluid viscosity while the shear rate remains constant. Fluid viscosity was increased up to 3X seawater by the addition of PVP-360, and experiments were performed with the dinoflagellate *Lingulodinium polyedrum* (=*Gonyaulax polyedra*) according to our standard protocol for pipe flow studies.

<u>Effect of agitation on cell morphology.</u> UCSD undergraduate student Marnie Zirbel has studied the change in morphology of the oligotrophic dinoflagellate *Ceratocorys horrida* when subjected to agitation on an orbital shaker. The change in cell morphology was monitored using light, scanning electron, and laser confocal microscopy. Population growth rates were measured, and the sinking rate of individual cells was measured from high magnification video recordings. This dinoflagellate exhibits major yet reversible changes in cell size and spine length due to agitation.

<u>Interspecific variation in the response to shear.</u> Shear acts directly on dinoflagellates by inhibiting cell division, thus affecting population growth (long-term effect), and by stimulating bioluminescence (short-term effect). Graduate student Nauen has studied shear sensitivity in five species of cultured dinoflagellates. The instantaneous response was studied by measuring bioluminescence stimulated by fully developed pipe flow. The qualitative effect on net population growth was monitored in cells grown under active hydrodynamic conditions on an orbital shaker.

<u>Bioluminescence as a flow diagnostic</u>. Noever and Cronise (1994) have reported that bioluminescence can be used to resolve eddy structure in turbulent flow. In collaboration with Jeff Allen, a mathematician at NRaD, we performed an experimental and mathematical study which refutes their conclusions. The study tested the following hypotheses: turbulent flow is necessary to stimulate bioluminescence, the response is affected by the length scales of the flow, and the amplitude density of bioluminescence events is directly related to the distribution of turbulent length scales in the flow.

WORK COMPLETED

Response to shear and acceleration. The experimental phase of the study has been completed, and the computational work is near completion.

Mechanism of the shear response. The experimental phase of the project has been completed, and all data have been analyzed. A manuscript is now in preparation.

Effect of agitation on cell morphology. The experimental phase of this study has been completed, and a manuscript is near completion.

Interspecific variation in the response to shear. The experimental phase of this study has been completed, and a manuscript is currently in preparation.

Bioluminescence as a flow diagnostic. The study has been completed, and a manuscript published (Rohr et al. 1997).

RESULTS

Response to shear and acceleration. In fully developed laminar and turbulent pipe flow, the response of the red tide dinoflagellate *Lingulodinium polyedrum* (=*Gonyaulax polyedra)* is best correlated with shear. In flows near the response threshold, cells which respond are located near the pipe wall, where the shear is greatest. Threshold response occurs at shear stress levels of approximately 0.2 N m^{-2}. In the developing (accelerating) flow of a convergence, cells respond only near the wall at a downstream position where the boundary layer is developing and appreciable levels of shear stress are present. Cells respond only in the region where the level of shear stress is greater than the response threshold based on fully developed pipe flow. Initial results were presented at the 1997 ASLO meeting, and final results will be presented at the 1998 Ocean Sciences meeting.

Mechanism of the shear response. When dinoflagellates respond to fluid shear, are they sensitive to the force acting on them (shear stress) or the rate of strain acting across the cell (shear rate)? Graduate student Jennifer Nauen has examined this aspect of bioluminescence stimulation by manipulating the fluid viscosity in fully developed pipe flow. Because shear stress = shear rate x viscosity, the shear stress acting on the cell can be increased by increasing the fluid viscosity while the shear rate remains constant.

The luminescent response was independent of viscosity, indicating that cells respond according to shear stress and not shear rate. This is similar to results for protozoa and animal cells, which also respond to mechanical stimulation via a shear stress mechanism. Nauen's results, the first study of the nature of the shear mechanism for any planktonic organism, were presented at the 1996 Ocean Sciences meeting.

Effect of agitation on cell morphology. UCSD undergraduate student Marnie Zirbel has determined that the oligotrophic dinoflagellate *Ceratocorys horrida* exhibits major yet reversible changes in cell size and spine length due to turbulence. Normally long-spined cells become smaller in size with greatly reduced spines when maintained under active hydrodynamic conditions. This effect is completely reversible. Under transfer to quiescent conditions, cells revert to the large long-spined condition. We speculate that the ecological

importance of the morphology change is to protect the cell from mechanical damage; in turbulent conditions the long spines are unnecessary to reduce sinking. This appears to be the first demonstration of a short-term reversible morphological effect of “turbulence” for any planktonic organism.

<u>Interspecific variation in the response to shear.</u> Shear sensitivity is not related to cell size or the presence of thecal plates. Based on bioluminescence stimulation experiments with fully developed pipe flow, the long-spined species, *Ceratocorys horrida*, is one order of magnitude more sensitive than the other species. The spines may act as levers to exaggerate the effect of the local shear. Unlike red tide dinoflagellates, non-red tide species are able to grow under active hydrodynamic conditions. The physiological basis of shear sensitivity is yet to be determined. Our initial results were presented at the 1996 Ocean Sciences meeting, and a manuscript will be completed during fall 1997.

<u>Bioluminescence as a flow diagnostic</u>. The threshold for flow-induced bioluminescence always occurs in laminar flow. Therefore turbulent flow is not required for bioluminescence stimulation. In laminar flow there are no length scales; furthermore, the maximum organism response occurs in high laminar flow, and transition to turbulence did not cause an additional response. Analysis of the amplitude density of flash events does not discriminate between laminar and turbulent flows. These results (Rohr et al. 1997) refute the conclusions of Noever and Cronise (1994), and clarifies the laminar and turbulent conditions which stimulate plankton bioluminescence. We have applied these results to the flow field around a dolphin (Rohr et al. in revision) and an aircraft carrier.

We are beginning to determine what information can be obtained directly from the bioluminescence time series. Numerical models, with known assumptions, are being developed to generate bioluminescence time series to compare with experimental data. This will help us determine: (1) whether bioluminescence time series can be used to differentiate laminar from turbulent flow, (2) the distribution of turbulent length scales, (3) different species response to flow agitation, and (4) the best estimate of flash decay.

IMPACT

Based on the results of this study, we conclude that: (1) the stimulation threshold for bioluminescence occurs in laminar flow for a variety of flow fields, (2) the response is best correlated with shear stress, with the maximum cell response occurring in high laminar flow; no further increase in light emission per cell occurs upon transition to turbulent flow, (3) for flow around moving objects of interest to the Navy, the amount of stimulated bioluminescence is a function of boundary layer thickness and the degree of flow separation.

During 1997 a meeting on Oceanographic Support for Special Warfare was held in San Diego. One of the goals of the conference was to identify oceanographic parameters which impact the operations of Naval Special Warfare (SPECWAR) missions. One major concern of nighttime covert operations is the potential exposure from bioluminescence stimulated by swimmers or swimmer delivery vehicles. The conference draft report includes recommendations for obtaining critical data on bioluminescence which can be integrated into mission planning. Our research on the hydrodynamic stimulation of plankton bioluminescence directly addresses these needs.

TRANSITIONS

It is hoped that the applied significance of the results of this study can be useful to nighttime mission planning (see Impact section).

RELATED PROJECTS

This project benefits greatly from collaboration between Latz of SIO and Rohr of NRaD, San Diego. Our successful blend of physics and biology has revealed new insights into the effect of flow on planktonic organisms.

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A description of research activities, including a summary of the present project with a list of recent publication and abstracts, can be found at the laboratory Web site at http://siolibrary.ucsd.edu/mlatz/. The Web site has been very popular -- during August 1997 it received more than 300 independent “hits”, representing 21 foreign countries in addition to the U.S.